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APPLICATION NUMBER: ***60/556,585***
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SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

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PTO/SB/16 (10-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 381 798 175 US

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[X] Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

LONG LASTING INSULIN DERIVATIVES AND RELATED METHODS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification *Number of Pages* 28 [ ] CD(s), Number
[ ] Drawing(s) *Number of Sheets* [X] Other: RETURN RECEIPT POSTCARD
[X] Application Data Sheet. See 37 CFR 1.76 - 3 pgs

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.
[ ] A check or money order is enclosed to cover the filing fees
[X] The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-1952

FILING FEE AMOUNT ($): 80.00

[ ] Payment by credit card. Form PTO-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

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Date March 25, 2004

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REGISTRATION NO. *(if appropriate)* 38,651

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Docket Number: 500863003600

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 381 798 175 US in an envelope addressed to: Box Provisional Patent Application, Commissioner for Patents, Alexandria, VA 22313-1450, on the date shown below.

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number	500863003600

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SF-1669858

Application Data Sheet
Application Information

ATTORNEY DOCKET: 500863003600

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Subject Matter::	**Utility**
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CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	None
Title::	**LONG LASTING INSULIN DERIVATIVES AND RELATED METHODS**
Attorney Docket Number::	**500863003600**
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	None
Small Entity?::	**YES**
Petition Included?::	No
Secrecy Order in Parent Appl.?::	No

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Domestic Priority Information

Application::	Continuity Type:	Parent Application::	Parent Filing Date::
N/A			

Foreign Priority Information

Country::	Application Number::	Filing Date::	Priority Claimed::
N/A			

PATENT APPLICATION SERIAL NO. _____

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FEE RECORD SHEET

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PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-267/60033

TITLE :

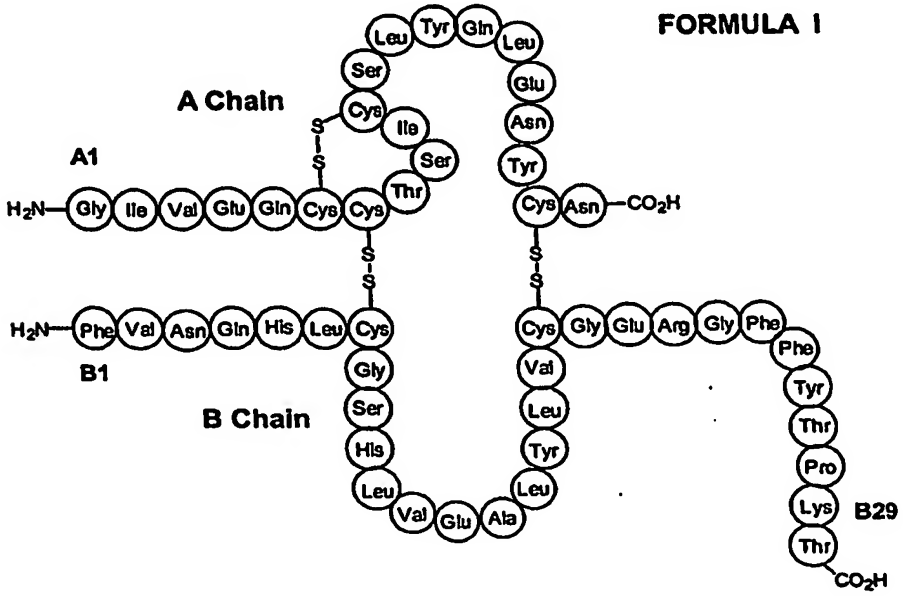
Long Lasting Insulin Derivatives And Related Methods

DESCRIPTION OF THE INVENTION :

The invention relates to a long lasting insulin derivative. More particularly, the insulin derivative comprises an insulin molecule and a reactive group coupled thereto, the reactive group being capable to covalently bond a blood component *in vivo* or *ex vivo*.

The insulin molecule may be native human insulin (see the sequence of native human insulin below in Formula I) or an analogue thereof such as an insulin molecule with amino acid substitution(s), amino acid deletion(s) or amino acid addition(s). The following are listed as examples of insulin analogue that can be used in accordance with the present invention: insulin glargine called Lantus® of Aventis Pharmaceuticals Inc., which has a glycine substituted in position A21 and two residues of arginine added in C-terminus of the chain B; insulin detemir called Levemir® of Novo Nordisk A/S, which is a native human insulin where threonine in position B30 is deleted and tetradecanoyl is added on the lateral chain of lysine B29; insulin lispro called Humalog® of Eli Lilly, which is Lys B28, Pro B29 human insulin; insulin aspart called NovoLog® of Novo Nordisk A/S, which Asp B28 human insulin; and insulin glulisine called Apidra® of Aventis, which is Lys B3, Glu B29 human insulin.

FORMULA I



The reactive group may be coupled to different functionalities on the insulin molecule or analogue thereof. According to preferred embodiments of the invention, the reactive group is coupled to an available amino group of the insulin molecule, such as the α-amino groups of the N-terminus amino acid of chains A and B, or the ϵ-amino group of Lys B29. In accordance with the invention, insulin analogue containing substituted and/or added amino acid(s) may contain additional amino group for coupling the reactive group; or other functionalities appropriate for coupling the reactive group thereto. Preferred reactive groups capable to covalently bond a blood component *in vivo* or *ex vivo*, are succinimidyl-containing groups and maleimido-containing groups. The more preferred reactive group is a maleimido-containing group, and more particularly MPA.

Optionally, the reactive group is optionally coupled to the insulin molecule via a linker. The linker is preferably selected from the group consisting of hydroxyethyl motifs such as (2-amino) ethoxy acetic acid (AEA), ethylenediamine (EDA), amino ethoxy ethoxy succinimic acid (AEES), 2-[2-(2-amino)ethoxy)] ethoxy acetic acid (AEEA), AEEA-AEEA, $-NH_2-(CH_2)_n-COOH$ where n is an integer from 1 to 20; one or more alkyl chains (C1-C10) motifs such as glycine, 3-aminopropionic acid (APA), 8-aminooctanoic acid (AOA), 4-aminobenzoic acid (APhA). Examples of combinations of linkers include, without limitations, AEEA-EDA, AEEA-AEEA, AEA-AEEA and the like. The preferred linker is AOA or the use of no linker with the reactive group MPA.

The present invention also relates to an insulin conjugate. The conjugate comprises an insulin derivative where its reactive group has reacted with a blood component *in vivo* or *ex vivo* so as to form a covalent bond. Therefore, the conjugate may be formed *in vivo* by the administration of the insulin derivative, or *ex vivo* by contacting the insulin derivative to a blood solution or purified blood components *in vitro* in conditions that allow formation of the covalent bond. Purified blood components can be provided by extraction and purification from blood sample or produced by recombinant techniques. The preferred blood component is a blood protein, and more preferably, serum albumin.

The present invention further relates to method for treating glycaemic-related diseases or disorders, comprising the administration of insulin derivatives or insulin conjugates being prepared *ex vivo*. Of course, glycaemic-related diseases or disorders include diabetes of Type I and II, and gestational diabetes. Also, cystic fibrosis,

polycystitc ovary syndrome, pacreatitis and other pancreas-related diseases may also be treated by the administration of insulin derivatives or insulin conjugates of the present invention. Insulin is also known as a growth factor and therefore, the insulin derivatives or insulin conjugates of the present invention can be useful in topical administration for wound healing and other related indications.

Division of Medicinal Chemistry Poster # 317

Regio-Selective Synthesis and In Vivo Evaluation of Insulin-Albumin Conjugates

A. Boutros, X. Huang, C. Soucy, V. Paradis, K. Thibaudeau, M. Robitaille, R. Léger, P. van Wyk, O. Quraishi, N. Bousquet-Gagnon and D. Bridon.

ConjuChem Inc., 225 President Kennedy Ave., Montréal, Québec, Canada, H2X 3Y8. www.conjuchem.com

Introduction

Insulin (**1**) is a vital endocrine hormone that binds to a cellular surface receptor setting off a cascade of events culminating in glucose absorption from the blood[1]. Low levels of insulin lead to severe disorders such as types I and II diabetes. Type I diabetes is a life threatening disease where the patient must self-administer quick acting insulin for survival. In Type II diabetes, the objective is glycaemic control to reduce the onset of long-term consequences, therefore treatment with insulin becomes necessary after the failure of lifestyle changes or hypoglycaemic drugs. When treatment with insulin is required, a long lasting form of insulin will lead to better glycaemic control and reduce the amount of injections resulting in increased patient appreciation and compliance.

There are several known "long lasting" insulin drugs that function through various modes. Some examples include the slow release from the injection site[2] or non-covalent binding to blood proteins through lipophilic interactions[3].

We have demonstrated that the bioconjugation of maleimido derivatives of peptides to Cys34 Human serum albumin (HSA) can prolong their presence in plasma by protecting them against elimination through metabolic or excretion pathways[4-7]. We became interested in the application of this methodology to insulin.

Insulin is a small protein consisting of two peptide chains with three disulfide bonds as seen on figure 1. The challenge was to use insulin as a starting material and selectively attach a single maleimido group to one of the three available amines.

We report herein the synthesis and characterization of maleimido derivatives of insulin. The structure activity relationship (SAR) of the resulting derivatives is assessed in a diabetic rat model.

Albumin: The Most Abundant Plasma Protein

Albumin

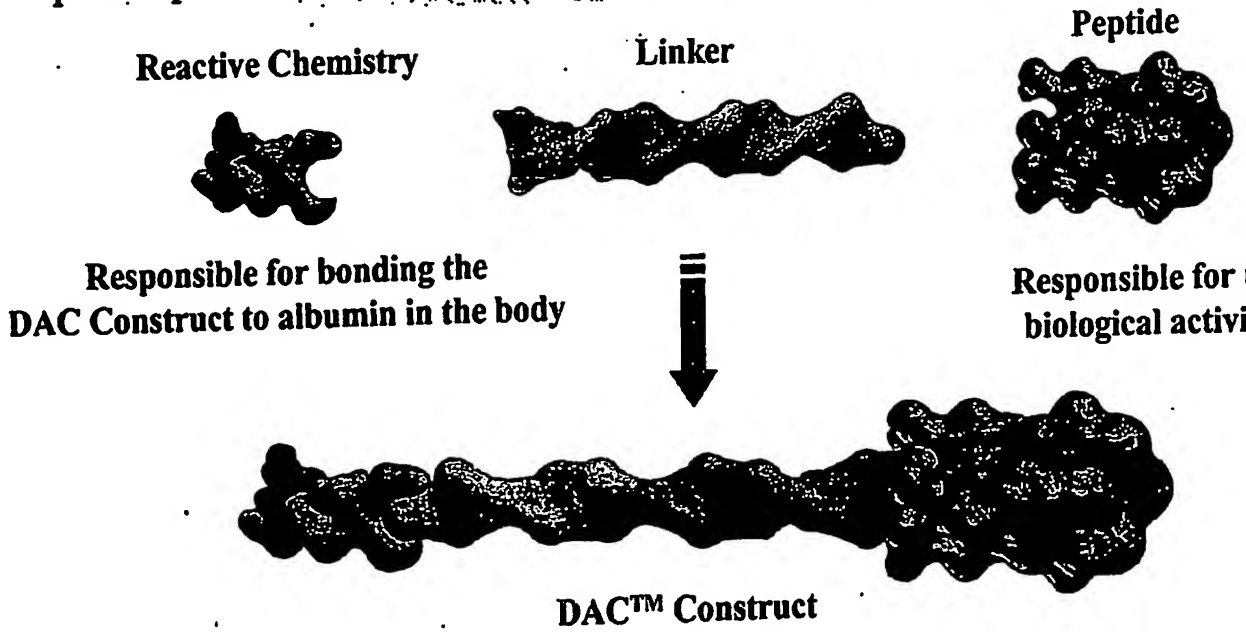
- ⇒ Plasma concentration 42 mg/mL (636μM)
- ⇒ Plasma half-life is species-dependent ; 14-20 days in humans
- ⇒ Molecular weight: 66450 (Human)

Cys34 of Albumin (~40% is capped)

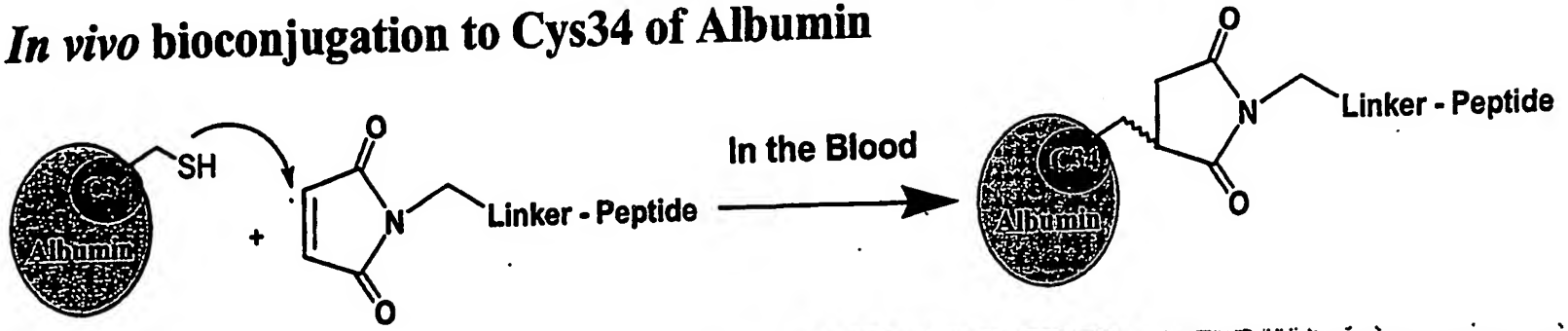
- ⇒ The free SH on albumin represents the larger portion of mercaptan found in plasma.
- ⇒ The unusual pKa of ~ 5.0[8c] for the SH of Cys34, makes it far more acidic than other thiol containing molecule like cysteine (pKa ~8.5)[8b] and Gluthatione (pKa ~ 8.9)[8b]
- ⇒ Sits in a hydrophobic pocket
- ⇒ S^- form at physiological pH

Drug Activity Complex (DAC™)Technology

Once bound, the DAC™ construct adopts a similar pharmacokinetic profile to the plasma protein to which it is attached while retaining the bioactivity of the original drug.



In vivo bioconjugation to Cys34 of Albumin



The coupling of DAC™ generates a stable covalent bond under physiological conditions. DAC™ enables a mild but highly selective addition onto Cys34 and in so doing prevents the rapid renal clearence usually associated with peptides while retaining pharmacodynamic profile of the original drug or peptide hormone.

Chemistry

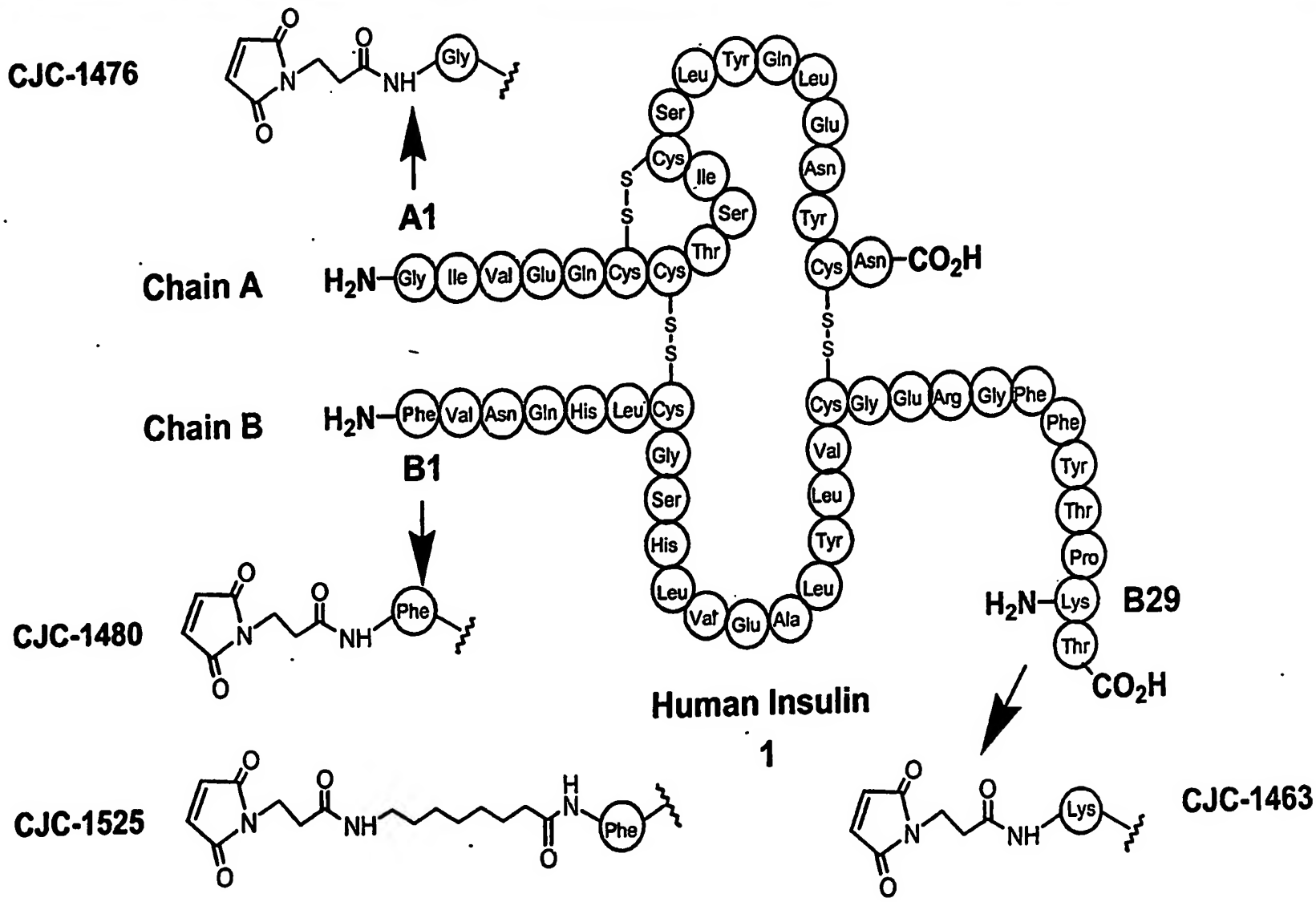


Figure 1

The Maleimide (MPA) functional group was found to react rapidly and selectively with HSA's unique free thiol via a Michael addition, forming a physiologically stable thioether bond.

Human insulin has three primary amino groups amongst its two chains namely the α-amino groups of Gly(A1) and Phe(B1), and the ε-amino group of Lys(B29). The attachment of a single MPA to the desired amino group of insulin was achieved by selective protection or a buffer system[9]. Characterization of the conjugates by LC/MS and N-terminal protein sequence analyses verified that a single MPA was attached to the selected residue of interest (Gly(A1), Phe(B1) or Lys(B29)).

Synthesis of MPA-N$^{\alpha}$-Gly (A1)-Insulin (CJC-1476)

Conditions favoring acylation at the amino group with the lowest pKa

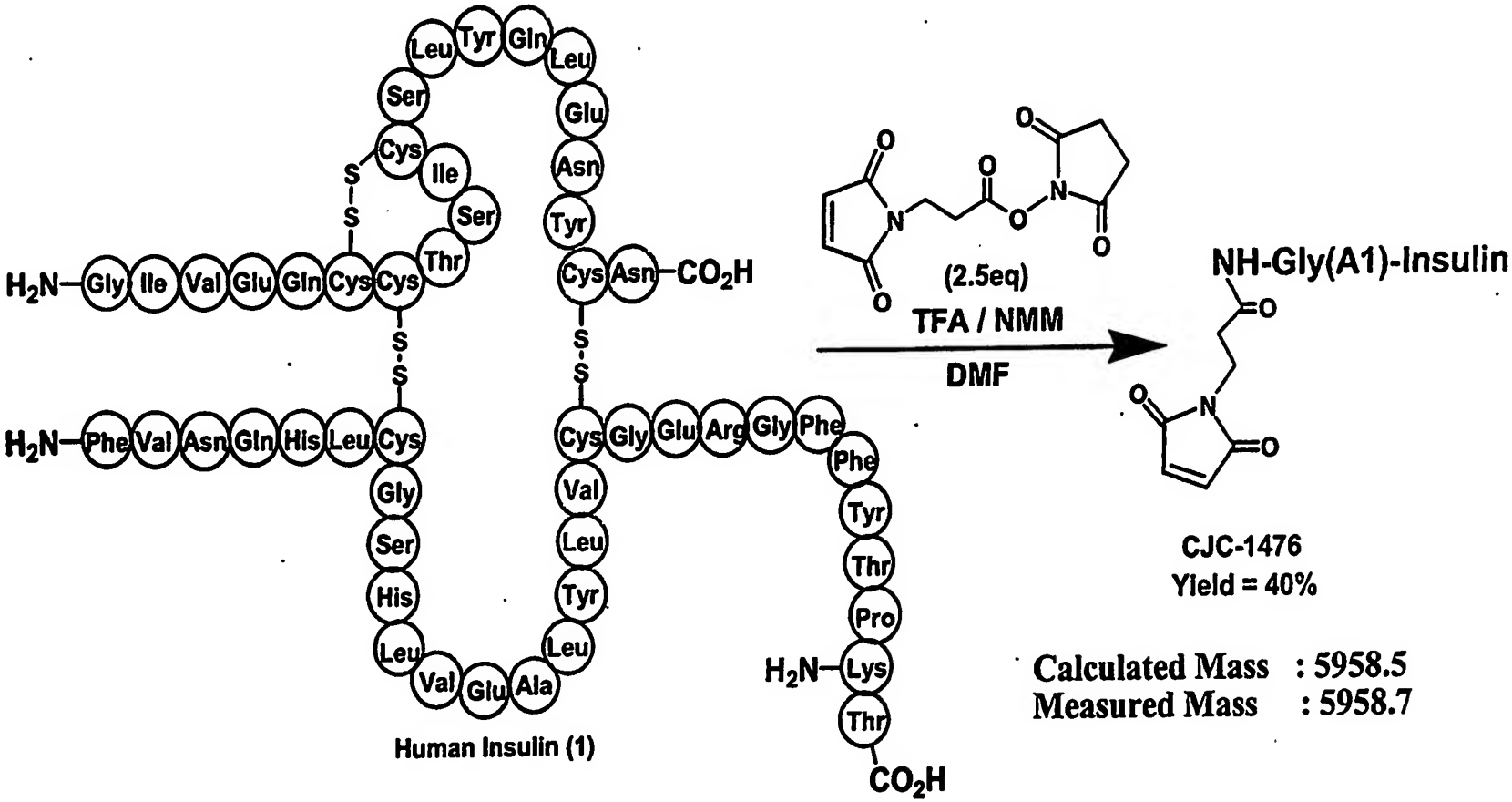


Figure 2

Synthesis of MPA-N$^{\alpha}$-Phe (B1)-Insulin (CJC-1480 and CJC-1525)

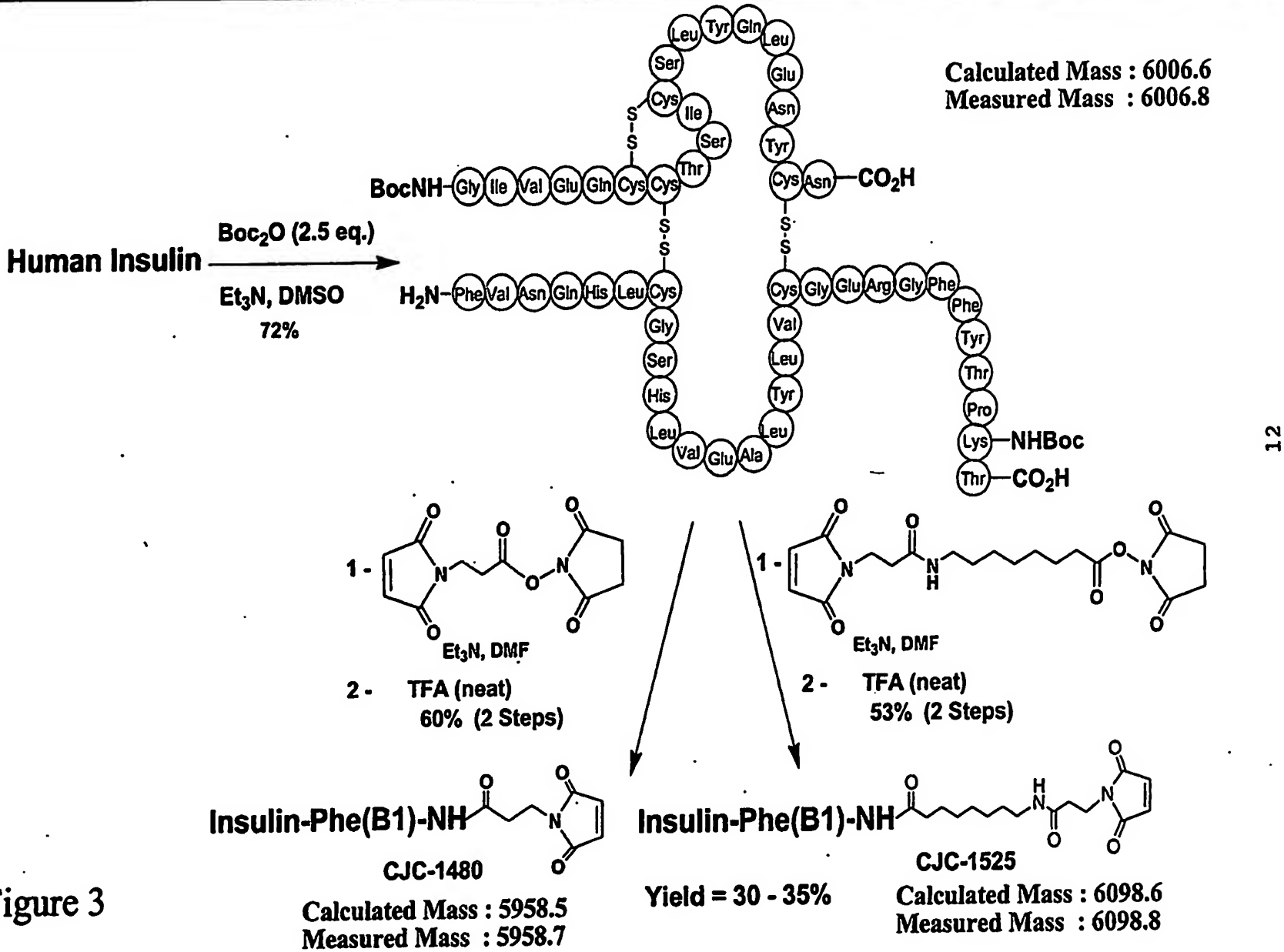


Figure 3

Synthesis of MPA-N$^{\varepsilon}$-Lys(B29)-Insulin (CJC-1463)

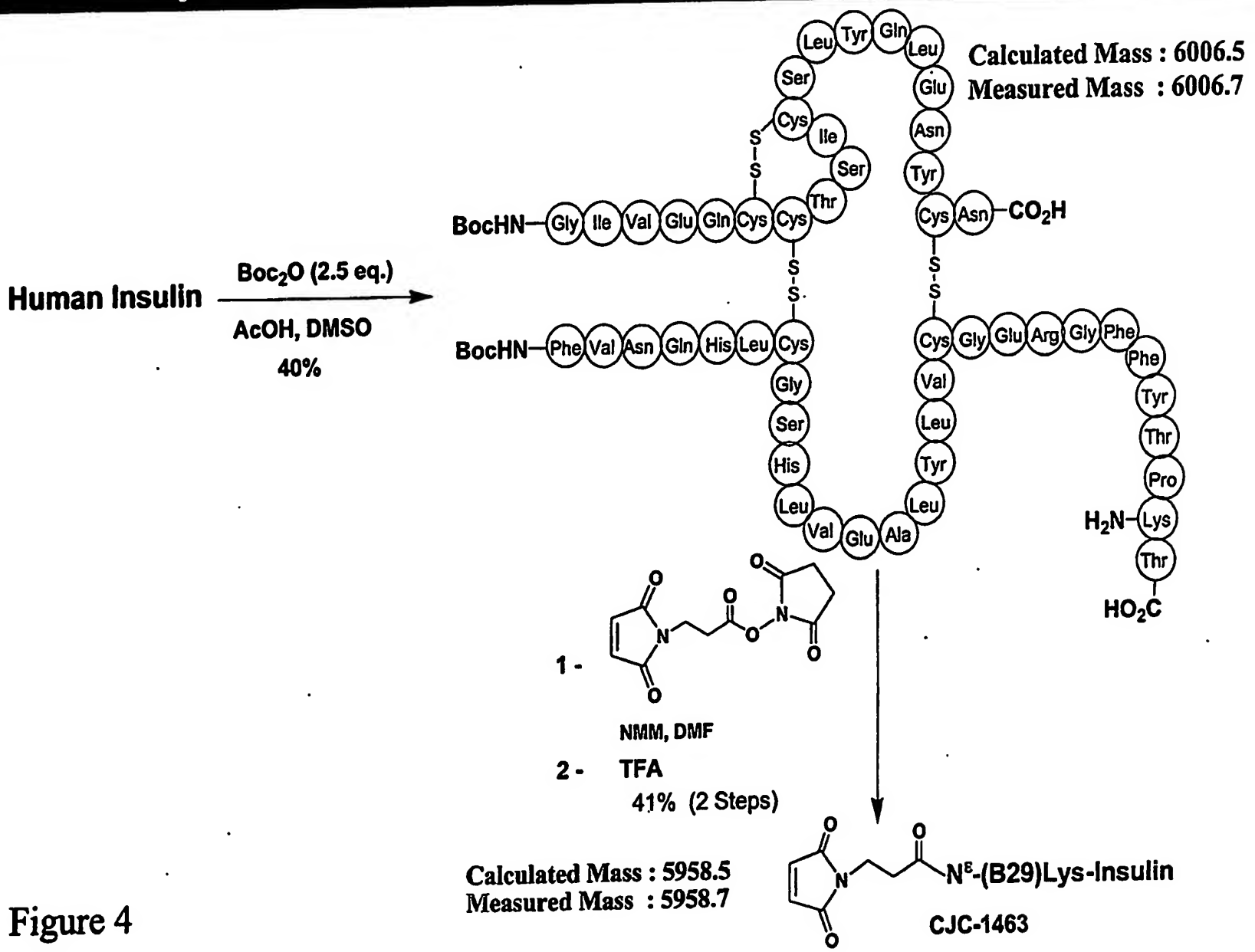
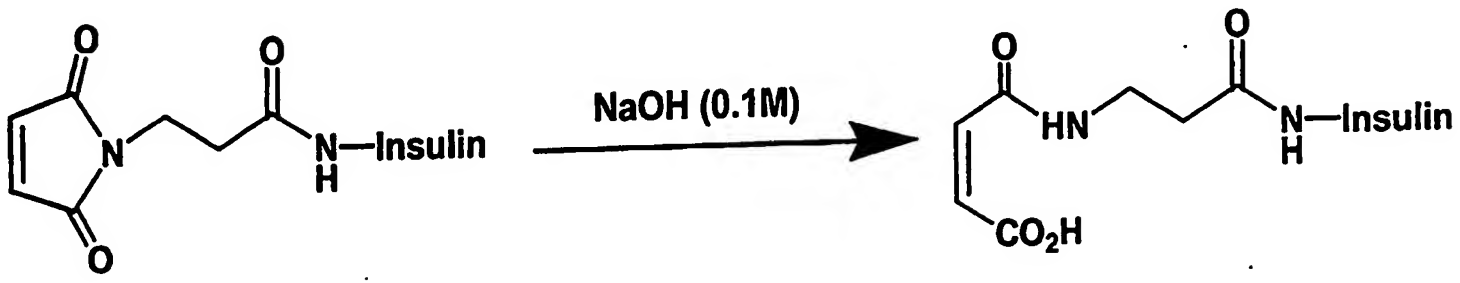


Figure 4

Hydrolysis of MPA-N$^{\alpha}$-Phe (B1)-Insulin

Hydrolyzed MPA-insulin derivatives were prepared for structure charaterization studies and to prevent an eventual chemical degradation in the Edman cycles.



Characterization of Hydrolyzed MPA-Insulin Derivatives

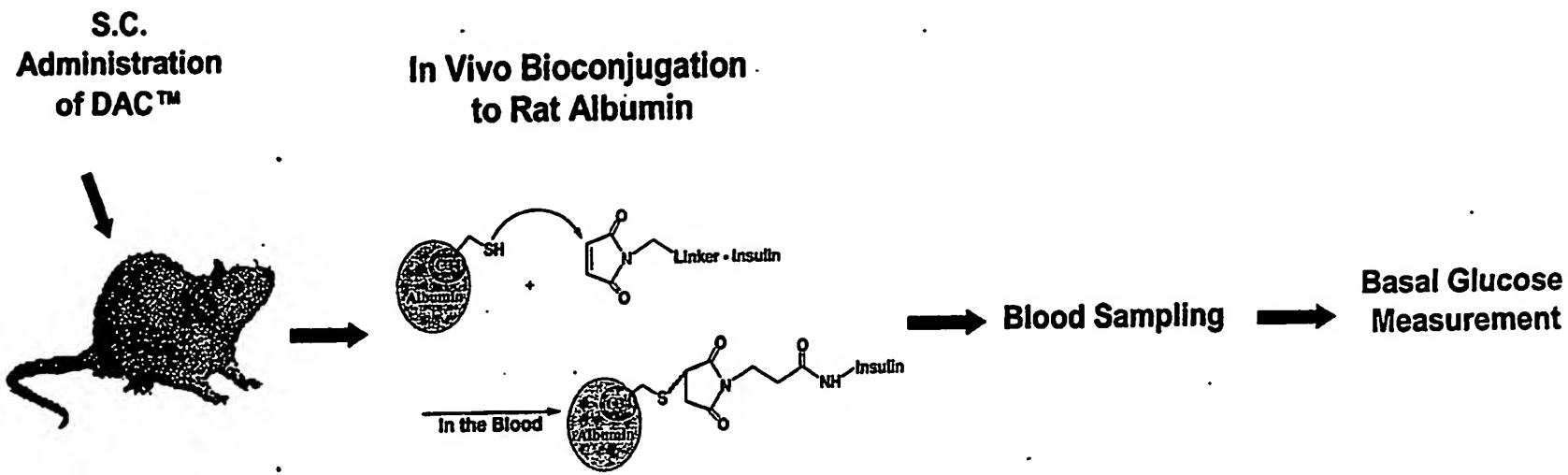
	Hydrolyzed CJC-1476	Hydrolyzed CJC-1480	Hydrolyzed CJC-1525	Hydrolyzed CJC-1463
Calculated Mass	5976.5	5976.5	6116.6	5976.5
Measured Mass	5976.7	5975.7	6117.8	5975.7

Edman Degradation of Hydrolyzed MPA-Insulin Derivatives

Compound	Chain	Edman Degradation Results (Positions)			
		1	2	3	4
Human	**A**	Gly	Ile	Val	Glu
Insulin	**B**	Phe	Val	Asn	Gln
Hydrolyzed	**A**	--	--	--	--
CJC-1476	**B**	Phe	Val	Asn	Gln
Hydrolyzed	**A**	Gly	Ile	Val	Glu
CJC-1480	**B**	--	--	--	--
Hydrolyzed	**A**	Gly	Ile	Val	Glu
CJC-1525	**B**	--	--	--	--
Hydrolyzed	**A**	Gly	Ile	Val	Glu
CJC-1463	**B**	Phe	Val	Asn	Gln

In Vivo Evaluation

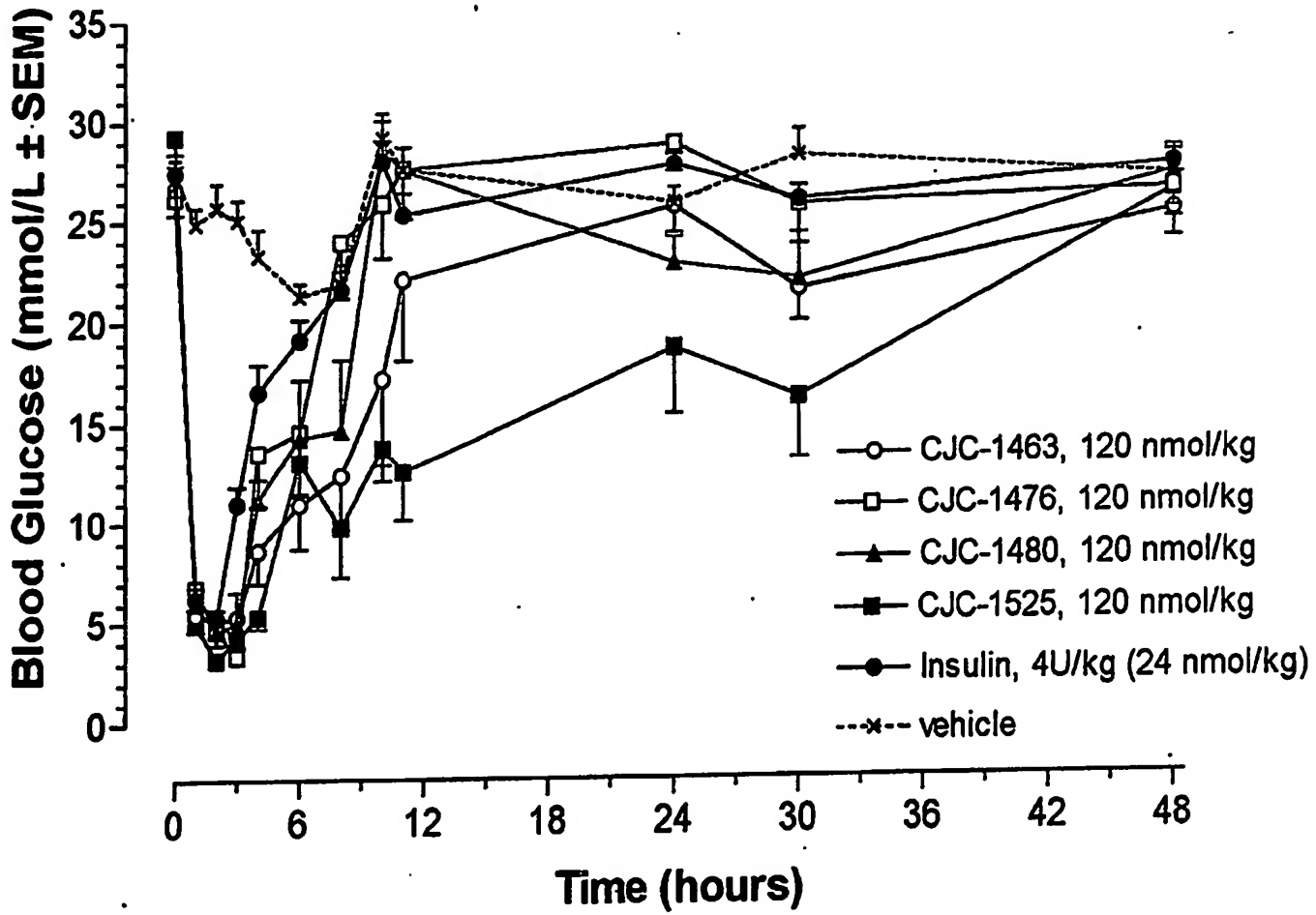
Evaluation in a streptozotocin-induced diabetes rat model is presented to demonstrate the biological activity of these different MPA-insulin derivatives.



⇒ **Streptozotocin-Induced Diabetic Rats**[10]

⇒ **Free Feeding Rats**

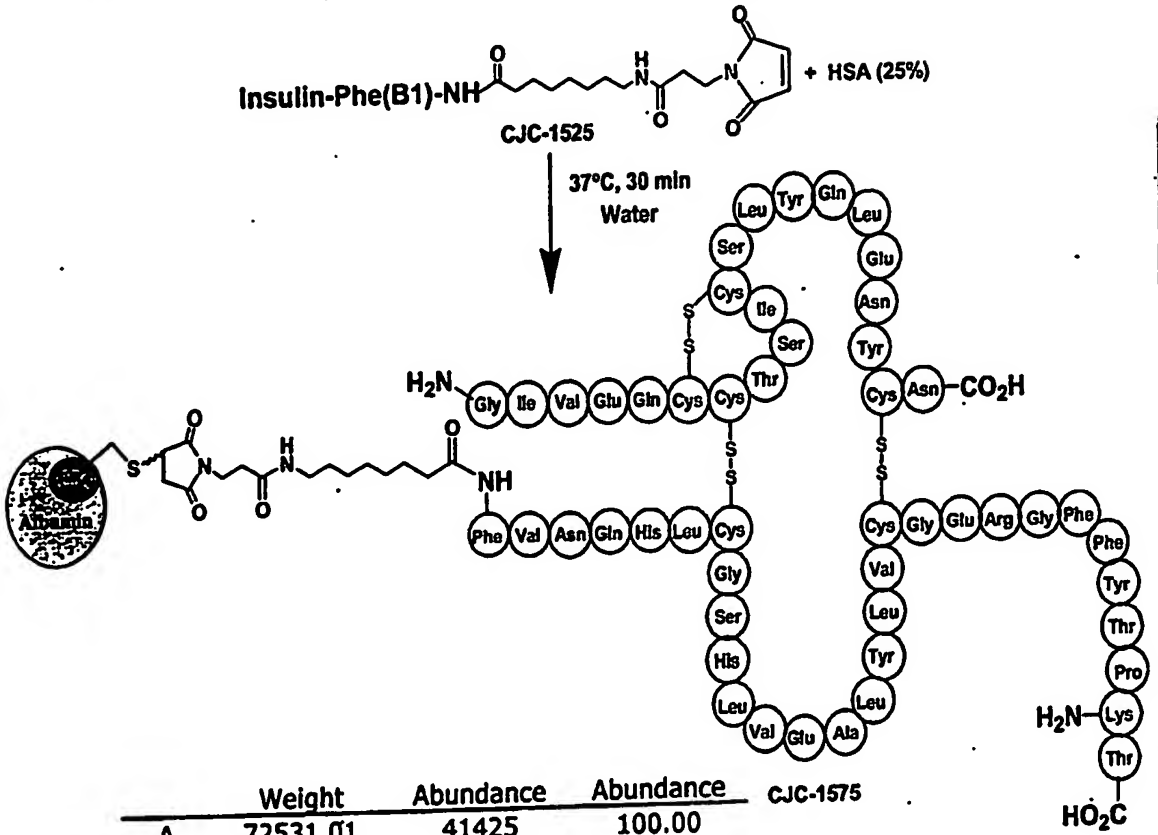
Pharmacodynamics of DAC™:Insulin Derivatives in Diabetic Rats



Diabetes was induced in male CD rats with a single IV injection of Streptozotocin (60 mg/kg). Two days later, rats received a single SC injection of DAC™:insulin derivatives at 120 nmol/kg, insulin at 4U/kg (24nmol/kg) or vehicle. Blood glucose levels were measured with a hand-held glucometer just prior injection and at 1, 2, 3, 4, 6, 8, 10, 11, 24, 30 and 48 hours post injection. 5 rats/group except for vehicle 3 rats/group.

Synthesis of Pre-Formed CJC-1525:HSA Conjugate

Preformed conjugate of CJC-1525 was synthesized as a control. CJC-1525:HSA conjugate (CJC-1575)



	Weight	Abundance	Abundance
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B	53001.75	7603	18.35
C	72625.84	7394	17.85
D	72692.1	7201	17.38
E	72344.84	5215	12.59
F	62839.67	3690	8.91
G	72789.02	3615	8.73
H	69271.08	2208	5.33
I	57094.48	1980	4.78

*******End of Report********

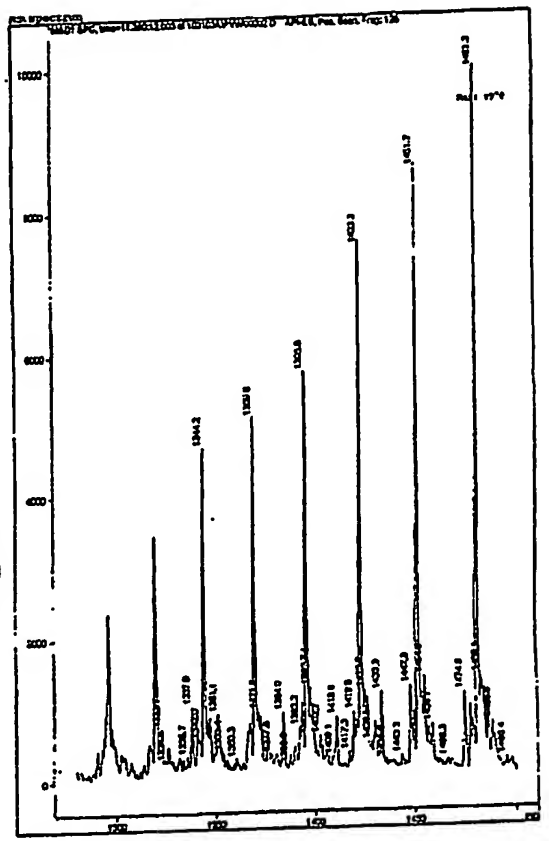
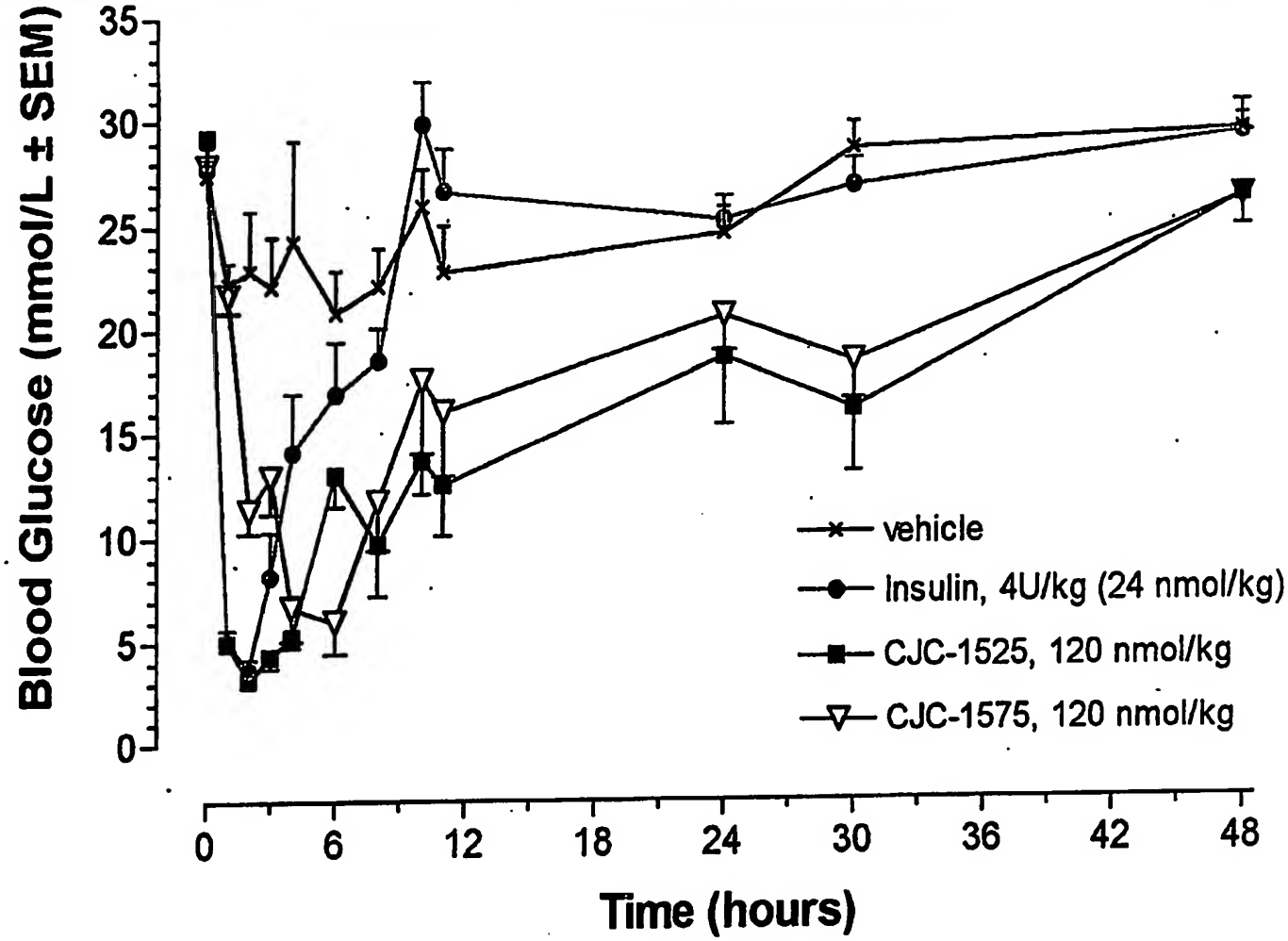


Figure 5

CJC-1525 *versus* CJC-1575



Blood glucose levels following a single SC injection of CJC-1525, CJC-1575, Insulin or vehicle in Streptozotocin-induced diabetic CD rats.

Conclusion

- Natural insulin normally has a short half-life[1] in higher order organisms.
-The conjugation of different MPA derivatives to specific residues of insulin was achieved using either selective protection or a buffer system.
-In these syntheses, we used a common Boc_2O protecting agent for the amino groups.
- All four derivatives showed blood glucose control in vivo over extended periods as compared to insulin.
- One Compound CJC-1525 showed extended duration of action in our rat model, both as a DAC™ and a preformed conjugate.

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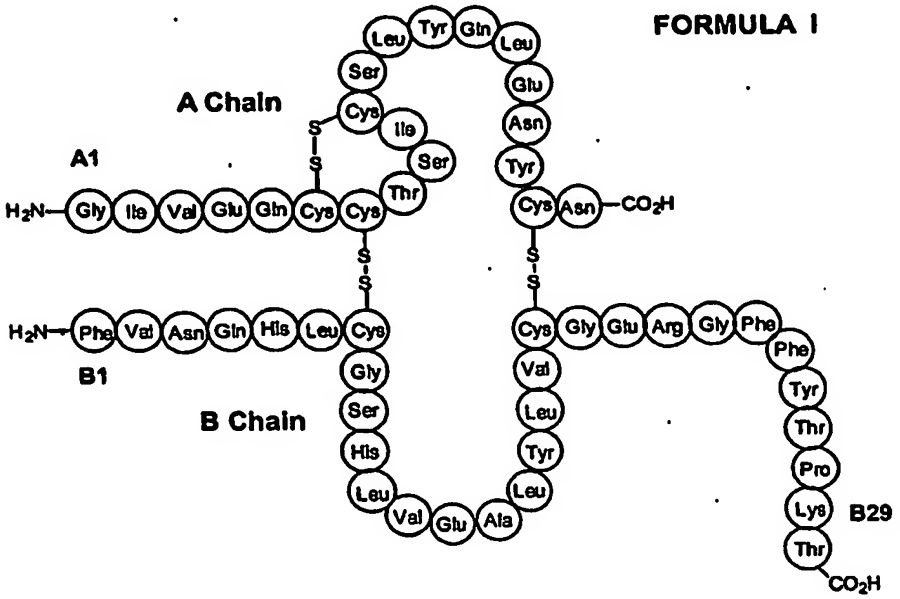
While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications, and this application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention, and including such departures from the present description as come within known or customary practice within the art to which the invention pertains, and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

CLAIMS :

1. An insulin derivative comprising an insulin molecule and a reactive group capable to covalently bond a blood component *in vivo* or *ex vivo*.

2. The insulin derivative of claim 1, wherein the insulin molecule is of formula I:



the reactive group being coupled to an amino acid of the insulin molecule, said amino acid being selected from the ones in positions A1, B1 and B29.

3. The insulin derivative of claim 1 or 2, wherein the reactive group selected from the group consisting of a succinimidyl-containing group and a maleimido-containing group.

4. The insulin derivative of claim 3, wherein the reactive group is a maleimido-containing group.

5. The insulin derivative of claim 4, wherein the reactive group is MPA.

6. The insulin derivative of any one of claims 1 to 5, wherein the reactive group is coupled to an amino acid of the insulin molecule via a linker; the linker being selected

from the group consisting of AEEA, AEEA-AEEA, EDA and -NH_2-$(CH_2)_n$-COOH where n is an integer between 1 and 20.

7. The insulin derivative of claim 6, wherein the linker is -NH_2-$(CH_2)_7$-COOH.

8. An insulin conjugate comprising an insulin derivative according to any one of claims 1 to 7, wherein the reactive group has reacted with a blood component *in vivo* or *ex vivo* so as to form a covalent bond.

9. The insulin conjugate of claim 8, wherein the blood component is a blood protein.

10. The insulin conjugate of claim 9, wherein the blood protein is serum albumin.

11. A method for treating a glycaemic-related disease or disorder, comprising the administration of the insulin derivative according to any one of claims 1 to 7.

12. A method according to claim 11, wherein the glycaemic-related disease is diabetes of type I or II.

13. A method for treating a glycaemic-related disease or disorder, comprising the administration of the insulin conjugate according to any one of claims 8 to 10, where the covalent bond was formed *ex vivo*.

14. A method according to claim 13, wherein the glycaemic-related disease is diabetes of type I or II.

15. Use of the derivative defined in any one of claims 1 to 7, for the preparation of a medicament for the treatment of a glycaemic-related disease or disorder.

16. Use according to claim 15, wherein the glycaemic-related disease is diabetes of type I or II.

17. Use of the conjugate defined in any one of claims 8 to 10, for the preparation of a medicament for the treatment of a glycaemic-related disease or disorder.

18. Use according to claim 17, wherein the glycaemic-related disease is diabetes of type I or II.

ABSTRACT

LONG LASTING INSULINN DERIVATIVES AND RELATED METHODS

The invention relates to a long lasting insulin derivative. More particularly, the insulin derivative comprises an insulin molecule and a reactive group coupled thereto, the reactive group being capable to covalently bond a blood component *in vivo* or *ex vivo*.